Question from Carolyn Friedman: *Describe the chemical reactions involved in ocean acidification.  How are key parameters (e.g. DIC, TA, pH, CO2(aq), carbonate saturation level, aragonite saturation) measured and what is the theory behind methods used in each measurement?*

Ocean acidification is an ongoing and insidious result of increasing amounts of greenhouse gases in the earth’s atmosphere since the onset of industrialization. From ~1750 the beginning of the 21st century, there has been a global average decrease of 0.1 pH units in the world’s oceans due to the uptake of anthropogenic carbon. By the end of the 21st century, the Intergovernmental Panel on Climate Change (IPCC) projects that there will be further decrease of 0.14-0.35 pH units, depending on the emissions scenario considered (IPCC 2007). There is no evidence that the ocean’s pH was more acidic than 0.6 units less than today’s pH over the past 300 million years, but some projections estimate decreases up to 0.77 pH units by 2300 (Caldeira & Wickett 2003). These projected changes can be viewed as short-term when put in the perspective of geological and evolutionary time. The ocean is only able to buffer CO2 changes that occur on a scale of greater than 105 years, however when changes in global CO2 occur on the timescale of less than 104 years the ocean is very sensitive and acidification occurs (Caldeira & Wickett 2003). The resilience of many ecosystems is likely to be overtaxed within the 21st century due to changes in global climate such as ocean acidification, temperature increases, pollution, land-use changes, and habitat fragmentation (IPCC 2007). This paper outlines the ocean chemistry changes that are specific to increases in anthropogenic CO2 and the parameters used to measure these changes.

Changes in the global ocean’s pH and carbon cycle can be caused by a number of factors, but input of anthropogenically sourced CO2 has a specific geochemical profile. Anthropogenic CO2 in the ocean increases the concentration of aqueous CO2, CO2(aq), and dissolved inorganic carbon (DIC) but does not change the charge balance or total alkalinity (TA) of seawater (Schulz et al. 2009). A greater concentration of CO2(aq) increases concentrations of bicarbonate,[HCO3-], and hydrogen ions, [H+], while decreasing carbonate, [CO32-], and the saturation state of the carbonate polymorphs aragonite and calcite (Schulz et al. 2009). These changes can be summarized in the equilibrium reaction:

CO2 + H2O  H+ + HCO3-  H+ + CO32- (**1**)

The chemical properties of CO2 and H2O that cause CO2(g) to be in equilibrium with ocean water is what spurs on ocean acidification. As more CO2 enters the atmosphere it dissolves in the ocean’s surface waters and prompts the production of H+ and HCO3- ions. The last step of the reaction, where H+ and CO32- are formed, is a slower step since CO32- is more thermodynamically stable bound to another ion; therefore, the major ions generated in this reaction are hydrogen and biocarbonate. In addition, as dissolved CO2 increases, additional CO2 reacts with CO32- to make more HCO3-, yielding more CO2 and HCO3- and less CO32- (Dickson in Riebesell et al. 2010). Due to the way this equilibrium lies, all IPCC emissions scenarios lead to undersaturation of aragonite in the surface Southern Ocean by 2100 and a global pH decrease from 0.14-0.35 pH units (Calderia & Wickett 2005; IPCC 2007). To measure the changes of the composition of CO2 in seawater, four parameters are needed: salinity, temperature, and a combination of two of DIC, TA, pH, or pCO2 (Dickson in Riebesell et al. 2010). The best choice of a combination of two parameters for the study of CO2-induced ocean acidification is 1. pH using a spectrophotometer and 2. total DIC using infrared (methods explained below; Dickson in Riebesell et al. 2010). DIC and pH allow an accurate description of the CO2 system without possible confounding parameters from other acid-base systems in seawater.

There is increasing evidence that ocean acidification is a contemporary as well as a future problem for the world’s oceans from increased CO2 emissions. Global atmospheric concentrations of CO2, methane (CH4), and nitrous oxide (N2O) are much greater now than they have been for thousands of years, mostly due to fossil fuel use and land-use changes resulting in greater amounts of CO2 (IPCC 2007). Global greenhouse gas (GHG) emissions have increased since the pre-industrial era, but the rate of increase is also getting faster: from 1970 to 2004 GHG emissions went up 70% (IPCC 2007). Carbon dioxide, the major component of GHG, has been increasing even more rapidly over the past two decades (IPCC 2007). The effects of increasing emissions are readily evidenced in the ocean waters. The highest concentrations of CO2 are in the near-surface waters (Sabine et al. 2004), the part of the ocean that is in direct equilibrium with the atmosphere. Ocean acidification in surface waters is proportional to increases in anthropogenic atmospheric CO2 supporting evidence of human-mediated pH change from the surface down to 150 m in the North Pacific (Byrne et al. 2010). With this rapid increase in ocean pH, the built-in carbonate buffering system may not be able to keep pace with the changes. If atmospheric CO2 continues to rise, the ocean pH will most likely fall at an accelerating rate (Byrne et al. 2010). At twice the pre-industrial amounts of atmospheric CO2 (preindustrial = 280 ppm CO2) there will be a 30% decrease in [CO32-] and a 60% increase in [H+], resulting in a decreased ability of the ocean to absorb more CO2 through loss of carbonate buffering (Sabine et al. 2004). The loss of available carbonate ions in the ocean will affect the saturation states (Ω) of both calcite and aragonite. There is already a shoaling of aragonite and calcite saturation horizons in the Atlantic, Indian, and Pacific Oceans due to a higher DIC:TA ratio caused by enrichment of DIC (Feely et al. 2004). Increase in DIC is direct evidence of heightened anthropogenic dissolution of CO2 into the oceans. To track and understand the effects of ocean acidification on the carbonate chemistry and acid-base balance of seawater, it is necessary to fully comprehend the chemistry and the measurement of that chemistry. In recent years, much effort has been put forth to produce better quality and standardized measures of DIC, TA, pH, and Ω. Verification of quality of DIC, TA, pH and fugacity of CO2 (*f*(CO2)) is important for comparison of results and there are currently standardized references available for all of these parameters (Dickson et al. 2007). The following will synthesize the chemistry and measurements of the key components in ocean acidification.

**Dissolved Inorganic Carbon (DIC)**

Dissolved inorganic carbon is the sum of the concentrations of the carbon-containing elements from Equation 1.

DIC = [CO2(aq)] + [HCO3-] + [CO32-] (**2**)

DIC is measured by acidifying a seawater sample and then extracting and measuring the unionized CO2 (Dickson et al. 2007). In the laboratory, manipulations that best mimic increased dissolved CO2 in the ocean are ones that change DIC while maintaining constant TA (Gattuso et al. in Riebesell et al. 2010). This can be achieved by aeration at the target partial pressure of CO2 (pCO2), addition of high-CO2 seawater, or addition of a strong acid and CO32- and/or HCO3- (Gattuso et al. in Riebesell et al. 2010). For aeration at a target pCO2, pH is monitored continuously while a controller opens and closes valves that deliver gas to seawater used in the experiment at levels according to the desired treatment. The gases in this method can be one of three combinations: 1. air + pure CO2, 2. CO2-free air + pure CO2, or 3. air, CO2-free air, and pure CO2. Carbon dioxide-free air is acquired using molecular sieves or CO2 scrubbers. For the addition of high-CO2 seawater, water is highly acidified with CO2 and then mixed with low-CO2 or ambient water. Since DIC and TA are conservative, the mixed water has parameters equal to the sum of the amounts in the two water sources. When adding a strong acid in combination with CO32- and/or HCO3-, the carbonate and bicarbonate ions elevate DIC and TA but the compensatory addition of acid balances the change in TA, mimicking the changes from CO2. DIC changes dependent upon changes in ionic concentration in the seawater, whereas TA varies according to changes in the overall charge\*ionic concentration (Gattuso et al. in Riebesell et al. 2010).

Once the method for changing the DIC in the ocean acidification experiment is implemented, measurements of DIC itself are the same for laboratory and ship-based. Seawater samples must be poisoned with mercuric chloride for DIC measurements so that no changes in DIC occur before or during analysis (Dickson et al. 2007). To extract the CO2 from the seawater, the water must be reacted with phosphoric acid in a borosilicate glass stripping chamber (Dickson et al. 2007). Coulometry is then used by passing an electric current through the sample to convert all of the CO2 to a different chemical state. Carbon dioxide is converted to a weak acid, hydroxyethylcarbamic acid via the reaction:

2CO2 + 2NH2(CH2)2OH  2HO(CH2)2NHCOOH (**3**)

Then a strong base, OH- ions, are electrogenerated by the reduction of H2O and used to titrate the weak acid. The equivalence point is detected photometrically with the indicator dye thymolphthalein, which turns from blue to colorless (Johnson et al. 1985). DIC is then calculated from the equation in Dickson et al. (2007):

DIC = ((Ns – *b*\**t* – *a*)/*c*)\*(1/(Vs\* ρ)) (**4**)

Where Ns is coulometer reading for the seawater sample; *a* and *b* are the acid blank and background level of the system, respectively; *c* is the coulometer calibration factor; *t* is the time required to measure the water sample; Vs is the volume of seawater sample at the temperature used; and ρ is the density of the sample.

In environmental sampling of ocean DIC, it is important for ocean acidification research to tease out the changes in DIC from anthropogenic sources and the changes due to natural background variability (Byrne et al 2010). For calculations of the contribution of anthropogenic CO2 to the ocean, the following are needed: the total carbon, alkalinity, dissolved O2, temperature, and salinity of the ocean sample; the age of the water; and the history of the atmospheric CO2 levels (Gruber et al. 1996). In calculations of total anthropogenic CO2 in the ocean (CO2ant), the contributions from background variability need to be eliminated. The first of these is the soft-tissue pump, which includes DIC contributions from photosynthesis, respiration, and biomineralization. Since a proportional amount of O2 is used for the C involved in this pump, the soft-tissue C contribution can be calculated from dissolved O2 (Gruber et al. 1996). The carbonate pump can be eliminated using the alkalinity distribution corrected for nitrate cycling. Lastly, corrections for O2 saturation concentration, alkalinity, pre-industrial concentrations of C, and water age/depth must be accounted for (Gruber et al. 1996). The amount of DIC that is left after eliminating all of the background variation is the CO2ant.

**Total Alkalinity (TA)**

Total alkalinity is the number of moles of hydrogen ion that is equivalent to the excess of proton acceptors over proton donors in 1 kg of seawater (Dickson et al. 2007). Many elements contribute to TA, however most of the nutrients are considered negligible in surface water measurements in the open ocean. Seawater contains a number of acid-base species beyond CO2, boric acid, and water, which are mostly derived from minor nutrients (Dickson in Riebesell et al. 2010). For the most part, these minor nutrients are in deeper waters, but can be upwelled. These minor nutrients are of concern in the lab and may confound the contribution of CO2 to TA (Dickson in Riebesell et al. 2010). The following equation includes the contributors to TA (Schulz et al. 2009):

TA = [HCO3-] + 2[CO32-] + [B(OH)4-] + [OH-] + [HPO42-] + 2[PO4-3] + [H3SiO4-] + [NH3] + [HS-] – [H3PO4] – [H+]free – [HSO4-] – [HF] (**5**)

Total alkalinity is not usually one of the measurements chosen for determination of changes in ocean acidification parameters, probably because it is less straightforward that those that rely solely on carbon. It is also not influenced by oceanic CO2 uptake (Schulz et al. 2009). It is, however, an important component to the ocean carbonate system and to the changes that affect aquatic life.

TA can be altered in the lab while maintaining constant DIC by the addition of strong acids and bases (Gattuso et al. in Riebesell et al. 2010). Addition of acid decreases TA and bases increase TA. Manipulations of TA can change specific carbonate chemistry parameters to isolate the particular DIC changes that are eliciting the response of interest in an experiment. TA can be altered in one of four ways that facilitate comparisons with similar changes in DIC: 1. [CO2] and *f*CO2, 2. [HCO3-], 3. [CO32-], Ω of calcite and aragonite, or 4. [H+] and pH (Schulz et al. 2009). Samples for TA analysis should be poisoned if not being measured right away (Dickson et al. 2007). TA is measured experimentally with an acid pontentiometric titration in either a closed or open cell. The sample is titrated with HCl to a pH-determined equivalence point measured by an electrode (Millero et al. 1993). The HCl solutions are made to have the same ionic strength as seawater. TA is determined from the proton balance at the alkalinity equivalence point from the following equation:

(V0\*TA – V\*N)/(V0+N) = [HCO3-] + 2[CO32-] + [B(OH)4-] + [OH-] + [HPO42-] + 2[PO4-3] + [H3SiO4-] + [NH3] + [HS-] – [H3PO4] – [H+]free – [HSO4-] – [HF] (**6**)

In the above equation (a variation in Eqn 5), V0 is the initial volume of the sample, N is the normality of the acid titrant, and V is the volume of the acid added (Millero et al. 1993). The final calculation of TA assumes that the nutrient-supplied compounds are negligible (Millero et al. 1993). Using a closed cell titration allows for the assumption that DIC remains constant throughout the process (Dickson et al. 2007). Open cell titration occurs in two stages: 1. the seawater sample is acidified to a pH of 3.5-4.0 and stirred to allow all CO2 to escape, then 2. the sample is acidified again to a pH of 3.0. In the open cell system, the calculations are based on the assumption that total DIC is approximately 0 at a pH of 3.0-3.5 (Dickson et al. 2007).

**pH**

The change in global ocean pH is one of the main concerns of ocean acidification and it is standard to monitor it continually in experiments. The familiar pH probe and standardization buffers have been deemed not sensitive enough or specific enough for seawater studies of acidification and have been replaced with buffers made in artificial seawater and measurements accomplished with electrodes or spectrophotometry. The total [H+] in seawater is approximately equal to the concentrations of all the free hydrogen ions and the concentration of HSO4- and pH is the negative log of total [H+]. When using an electrode to measure the pH, the standardization buffers should be made in artificial seawater (Dickson et al. 2007), although the difficulty in doing this has led some researchers to discover that traditional buffers lend a decent approximation of salt water pH (M. O’Donnell pers. comm.).

The spectrophotometric method is more precise than the electrode method and does not involve custom-made buffers. This method uses molecular properties of the pH indicator dye, meta-cresol (*m*-cresol) purple (Byrne et al. 2010). A small amount of dye is added to each sample and absorbance is measured at 434 and 578 nm as well as at a non-absorbing wavelength for the dye (730 nm) against a reference of pure seawater (Byrne et al. 2010). The wavelengths, 434 and 578 nm, correspond to the absorption maxima of the acid (HI-) and base (I2-) forms of *m*-cresol purple, respectively (Dickson et al. 2007). The spectrophotometer depends on the acid and base forms of the indicator having substantially different absorbance spectra. The dye (0.05-0.1 mL for absorbance peaks between 0.4 and 1.0) is added to the seawater sample in a cuvette and a double-beam spectrophotometer is used to measure the peaks (Dickson et al. 2007). The pH is then calculated based on absorbance for each wavelength, extinction coefficients, and the equilibrium constant function of salinity and temperature for *m*-cresol purple (Dickson et al. 2007). A correction to the calculation needs to be made for the addition of the acid-base dye to the sample.

**Partial Pressure of CO2 (pCO2)**

The amount of CO2 in seawater is the ultimate cause of ocean acidification and projected increases in atmospheric CO2 underline the importance of accurately measuring pCO2 in the ocean. The partial pressure of CO2 is the mole fraction of CO2 (xCO2) in air multiplied by the total pressure (p) (Dickson et al. 2007):

pCO2 = x(CO2)\*p (**7**)

The pCO2 can only be truly measured in a discrete sample of seawater. For measurements of pCO2, seawater samples must be poisoned with mercuric chloride prior to analysis. A volume of air with a known initial amount of CO2 and a known amount of seawater are maintained in a closed system under constant temperature and pressure. The water and air will reach equilibrium and a sample of the air can be analyzed using a gas chromatograph or infrared CO2 detector (Dickson et al. 2007). This method will work for pCO2 of 250-2000 ppm (Dickson et al. 2007). Measurements of pCO2 need to be calibrated with three calibration gases that have known concentrations of CO2 that span the range of measured values for the experiment (Dickson et al. 2007).

An alternative method for measuring, or approximating, pCO2 is by measuring the fugacity of CO2 in its gas phase, or *f*CO2. Measuring *f*CO2 as opposed to pCO2 recognizes the non-ideal nature of the gas phase (Dickson et al. 2007). The fugacity coefficient is the ratio of fugacity to partial pressure and is a function of temperature and the gas phase composition. Fugacity is measured over pCO2 when the CO2 in the air is in equilibrium with a continuous stream of seawater and is only suitable for measurements between 250 and 550 ppm. A fixed volume of air is equilibrated with a continuous (~infinite) stream of seawater that goes through an equilibrator (Dickson et al. 2007). The CO2 content of the air will adjust to reach equilibrium with the seawater, at which point the air is sent through a non-dispersive infrared analyzer to measure CO2 (the analyzer having been calibrated with calibration gases; Dickson et al. 2007).

**Calcite and Aragonite Saturation (Ωc and Ωa)**

Calcite and aragonite are polymorphs of calcium carbonate and important structural components in the shells and exoskeletons of many marine invertebrates. Aragonite is 1.5 times more soluble than calcite and a third polymorph of CaCO3, magnesian calcite, has an intermediate solubility between the other two (Dickson in Riebesell et al. 2010). The saturation states (Ω) of calcite and aragonite are of particular concern in ocean acidification. As Ω decreases in seawater, there is less calcite or aragonite available for those organisms that need it structurally to survive. This is evidenced by the equation:

Ω = [Ca2+][CO32-]/Ksp (**8**)

The concentration of Ca2+ is estimated from salinity and [CO32-] is calculated from DIC and TA (see Eqns. 2 and 5; Feely et al. 2004). In brief, as atmospheric and CO2(aq) increase, CO32- becomes less available in the ocean to form calcite and aragonite, decreasing Ω for both. The concentration of carbonate ion is the main driver for changes in Ω (Feely et al. 2004). Ocean waters are considered saturated for a compound when Ω = 1, when Ω > 1 seawater is supersaturated and when Ω < 1 it is undersaturated. With evidence of aragonite Ω < 1 in a number of nearshore environments (Hauri et al. 2009; Feely et al. 2010), increased CO2ant and ocean acidification are already having effects on marine calcifiers.

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